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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/249,543	02/12/1999	THOMAS C. EVANS	NEB-154	1052

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EXAMINER
MOORE, WILLIAM W

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 05/20/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/249,543	Applicant(s) EVANS ET AL.	
	Examiner William W. Moore	Art Unit 1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 March 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,4-13,15-19,21-30,33-58,60 and 62* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,4-13,15-19,21-30,33-58,60 and 62* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Priority

Applicant's Amendment D, filed March 10, 2003, has been entered, amending page 1 of the specification to state Applicant's priority claim under 35 U.S.C. §119(e) to provisional application serial No. 60/102,413 filed September 30, 1998.

New Matter

The amendment filed March 10, 2003 is objected to under 35 U.S.C. §132 because it introduces new matter into the disclosure. 35 U.S.C. §132 states that no amendment shall introduce new matter into the disclosure of the invention. The added material which is not supported by the original disclosure is as follows: The amended claim 42 recites, "generating by *in vivo* synthesis, a first target protein fused to at least one first intein and a second target protein", constituting new matter where neither of the clauses, "generating by *in vivo* synthesis", and, "a first target protein fused to at least one first intein and a second target protein", are stated in the specification nor find any inherent support therein. Applicant is required to cancel the new matter in the reply to this Office Action.

Claim Objections

Claim 40 is objected to because of the following informalities: the word "plasmid" is stated in the singular at line 3 of the claim where the context requires the plural, "plasmids". Appropriate correction is required.

Sequence Compliance

Compliance with 37 CFR §1.821 is required in response to this Office action. Claims 1, 2, 4, 6, 11, 12, 18, 19, 22, 23, 25, 29, 30, 44, 45, and 47 do not have designations describing their subject matters, specifically, the amino acid sequence of the native *Mth* R1R1 intein, according to the requirements of 37 CFR §1.821 for a Sequence Disclosure. Even if the sequences were set forth in the claims, recitations of a nucleotide

or amino acid sequence positions must also include a statement of the designation, "**SEQ ID NO:n**", where "n" is an integer corresponding to the Sequence Disclosure. 37 CFR §1.821 also requires that sequence identifiers accompany descriptions of defined amino acid sequences in the specification with a designation properly stated as "**SEQ ID NO:n**". See 37 CFR §§1.821(b), (c) and (d). It is noted the disclosure of nucleic acid sequences encoding the native *Mth* R1R1 intein in Figure 4 of the specification provides an inherent disclosure of the encoded amino acid sequence that supports the filing of an amended Sequence Listing, both in computer-readable form and in printed form, that includes the amino acid sequence.

The printed form of the amended Sequence disclosure must be submitted as an amendment to the specification with a sentence that directs, i) deletion of the sequence listing filed as Amendment A, Paper No. 4 on September 14, 1999, and, ii) its replacement with the new sequence listing. A statement of identity of the contents of the paper and computer-readable copies of the sequence listing – a Statement in Support of Filing and Submissions in Accordance with 37 CFR 1.821(c) and (e) – must accompany the amendment. It is also noted that claims currently reciting modifications of the native intein amino acid sequence must be amended to correspond to the enumeration of the amino acid sequence actually encoded by the nucleic acid sequence set forth in Figure 4.

Response to Amendment

Applicant's amendments of Paper No. 14 to claims 1, 2, 4-9, 16, 17, 24, 34, 35, and 40-42, and cancellation therein of claims 3, 14, 20, 31, 32, 59 and 61, overcome the rejections of record of claims herein under 35 U.S.C. §103(a) over the teachings of various prior art patents. The claim cancellations of Paper No. 14 necessitate new grounds of rejection under 35 U.S.C. §112, second paragraph, and the incomplete nature of claim amendments in Paper No. 14 leave several original grounds of rejection under the first and

second paragraphs under 35 U.S.C. §112 outstanding. The claim amendments require the restatement of the obviousness-type double patenting rejection of record over claims now present in Applicant's copending application serial No. 09/786,009, and also alter the basis for the obviousness-type double patenting rejection of record of claim 1 herein over claim 96 in Applicant's earlier patent No. 5,834,247.

The claim amendments, together with journal publications of Böck et al. and Müller et al., submitted by Applicant and made of record herewith, and Hondal et al. made of record with Paper No. 10, mailed April 23, 2002, overcome several aspects of the rejections of record of claims herein under the first and second paragraphs of 35 U.S.C. §112. Specifically, the recitation "N-terminal cysteine or selenocysteine" in the amended claims 7, 8, 16, 17, 24, 40 and 42 more clearly describes the intended subject matter and is not a basis for a rejection under 35 U.S.C. §112, first paragraph, for the following reasons. With regard to enablement, Applicant establishes for the record that the disclosed method for intein-mediated ligation of first and second ligation partners can be practiced when either cysteine or selenocysteine is present at the amino-terminus of the second ligation partner, the partner that contributes the carboxyl-terminal region of the ligated product. Whether a second ligation partner is produced by solid-phase chemical synthesis or by expression as fusion polypeptide comprising an intein and a second ligation partner wherein subsequent cleavage of the fusion polypeptide at the amino-proximal junction between the second ligation partner and the intein releases the second ligation partner, either amino acid will permit ligation. Production of the second ligation partner as an independent polypeptide is facilitated by modification of the amino-terminus of the intein to which it was fused, when both were comprised by a recombinantly-expressed fusion polypeptide, so that splicing cannot occur by substituting serine or alanine for a cysteine at the amino terminus of the intein. Production of the first target intein is also

facilitated by recombinant modification of a native intein and, if necessary, the flanking extein which is the first target protein, by replacing the conserved asparagine at the carboxyl-terminus of the intein with an alanine to render the intein incapable of splicing but susceptible to cleavage at the second, intein-protein, junction.

The specification is considered to inherently provide an adequate written description that supports the amendatory recitation, "N-terminal cysteine or selenocysteine", in claims 7, 8, 16, 17, 24, 40 and 42 where it states, page 11, line 22, that "[t]he N-terminal residue [of the ligation partner that contributes the carboxyl-terminal region of a ligated product] may be any [ ] amino acid[] but preferably cysteine". Thus one skilled in the arts of molecular biology and protein synthesis and aware of the publications of Böck et al. and Müller et al., made before Applicant's provisional application was filed in September 1998, would consider that selenocysteine was also a naturally-occurring amino acid having characteristics sufficiently similar to cysteine to permit its substitution therefor, and that Applicant's disclosure included the recombinant insertion of the codon that - accompanied by a 3'-non-coding insertion signal in the transcribed mRNA - specifies selenocysteine at a position in a nucleic acid sequence encoding an intein-ligation partner fusion polypeptide just after the region encoding the carboxyl terminus of an intein incapable of splicing but susceptible to C-terminal cleavage. Such an artisan would appreciate that recombinant expression of the fusion polypeptide in an eukaryotic host cell followed by intein cleavage to release the second ligation partner would permit an amino-terminal selenocysteine in the second ligation partner to conduct a nucleophilic attack upon a carboxyl-terminal thioester of a first ligation partner according to the disclosed method.

This communication is not made final because new grounds of rejection for lack of enablement and/or lack of an adequate written description of certain method and product claims are also stated herein under 35 U.S.C. §112, first paragraph. These new grounds

of rejection are based on descriptive terms in the claims that are inadequate in view of the fact that the specification essentially discloses but a single method of protein ligation, inappropriately termed a "method for fusion" in claim 8, and improperly termed a "method for cyclic fusion" and a "method for polymerizing a plurality of target proteins" in, respectively, claims 16 and 17. While the specification describes accepting, or target, polypeptides that are amino-proximal exteins in extein-intein fusion polypeptides expressed in transformed host cells, it discloses that such extein-intein fusion polypeptides must be isolated from host cells in which they are expressed for *in vitro* cleavage by a thiol reagent at the extein-intein junction, thereby providing the carboxyl-terminal thioester of the first ligation partner or target polypeptide. The specification discloses no intermediate chemical species of a cleaved extein having a thioester that is subsequently contacted with a thiol reagent and does not disclose, discuss, or suggest, see e.g., pages 17-18, and page 19, the generic preparation of a first ligation partner or target polypeptide bearing a carboxyl-terminal thioester for subsequent ligation to a disclosed ligation partner other than by a thiol reagent-mediated, *in vitro*, cleavage from a carboxyl-proximal intein.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b). Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

This new ground of rejection is necessitated by Applicant's amendment of claim 1 in Paper No. 14. Claim 1 is rejected under the judicially created doctrine of obviousness-

type double patenting as being unpatentable over claim 96 of U.S. Patent No. 5,834,247, of record, in view of Smith et al., 1997, of record. Although the conflicting claims are not identical, they are not patentably distinct from each other because the subject matter of claim 1 in the instant application is embraced by claim 96 of the issued patent which recites a generic method "for synthesizing" a desired protein of which the method "for ligating" two proteins of claim 1 herein is an obvious species in view of the teaching of the *Mth* R1R1 intein depicted in Figure 8 of the November 1997 publication of Smith et al. The relationship between the genus of the patented claim 96 and the obvious species of the pending claim 1 herein is demonstrated by the description of claim 96 of the transformation of a recombinant host cell with a plasmid comprising a fusion gene wherein a region encoding a generic, "controllable", intein – which may be any intein, including the *Mth* R1R1 intein of Smith et al. – is fused in a common reading frame to a region encoding a target protein according to steps (a) and (b) of claim 96 and step (a) of claim 1 herein. The recombinant intein-target protein fusion polypeptide is then expressed in the host cell and next isolated from the host cell, steps (c) and (d) of claim 96, for *in vitro* cleavage, "contacting" in step (e) of claim 96, to produce a target protein having a carboxyl-terminal thiol ester, also known as a thioester, the same result as step (b) of claim 1 herein. Finally, steps (f) and (d) of claim 96 provide for the formation of a peptide bond between the target protein and another protein that has "an amino-terminal cysteine" which, according to disclosures of both the patent and the instant specification, is a "thioester reactive N-terminal amino acid" of claim 1 herein. Since the peptide bond formed in the patented claim is the result of the same transesterification reaction producing the ligated product of claim 1 herein, a patent bearing claim 1 herein issuing on the instant application would constitute an unjustified or improper timewise extension of the "right to exclude" granted by the patent because it would have been obvious to one of ordinary skill

in the art at the time the invention was made that the specific, *Mth* R1R1 intein, of Smith et al. is among the generic inteins of the patented claim, particularly when such an artisan at that time would have been motivated to use an *Mth* R1R1 intein of Smith et al. in view of the teaching of the issued patent, see col. 3, lines 25-33, that "reducing the overall size of the expressed [fusion polypeptide]" is "valuable" and the teaching of Smith et al. that the *Mth* R1R1 intein was the smallest intein yet known, permitting reduction in the size of a generic fusion polypeptide of clause (d) of the patented claim that comprises a generic intein. It is noted that method claims 2, 4-13 and 36-57 of the instant application are not subject to this rejection because all inherently require that a second ligation partner be recombinantly expressed as part of a protein-intein fusion polypeptide in a host cell, a process that is neither claimed nor taught by the patent.

Claim 35 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 96 of U.S. Patent No. 5,834,247 in view of Belfort, U.S. Patent No. 5,795,731, of record. Although the conflicting claims are not identical, they are not patentably distinct from each other because the subject matter of claim 35 in the instant application is embraced by claim 96 of the issued patent which recites a generic method "for synthesizing" a desired protein utilizing a "controllable intervening protein sequence", a term disclosed to include modifications of intein amino acids that flank an intein-extein junction to prevent extein splicing, wherein a process "for ligating" two proteins of claim 35 herein is obvious in view of the teaching of Belfort, Figure 2 and cols. 5-6, that modifying an intein by substituting an alanine for a native asparagine at the carboxyl-terminus of an intein renders it incapable of splicing due to lack of separation at its C-terminal intein-extein junction. Thus a patent issuing on the instant application with claim 35 would constitute an unjustified or improper timewise extension of the "right to exclude" granted by the patent because it would be obvious to one of

ordinary skill in the art at the time the invention was made that an intein bearing a substitution of alanine for a native asparagine at its carboxyl-terminus to prevent splicing according to Belfort is among the genus of "controllable intervening protein sequence[s]", of the patented claim, particularly when such an artisan at that time would have been motivated by both the teaching of col. 11, lines 55-57, of the 5,834,247 patent and the teaching of Belfort to make such alanine for asparagine substitutions because it they are efficacious in preventing splicing of flanking exteins, a necessary element of methods of the patented claim 96 and claim 35 herein.

Claims 1 and 35 are provisionally rejected, essentially for reasons of record, under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 22 and 28-33 of copending Application No. 09/786,009 in view of Smith et al., 1997, of record. This is a provisional double patenting rejection since the conflicting claims have not yet been patented. Although the conflicting claims are not identical, they are not patentably distinct from each other, in part because the methods of claims 1 and 35 herein, save for the use of a modified *Mth* R1R1 intein, are disclosed in the copending application and their subject matters are embraced by claims 22 and 28-32 of the copending application. Although the conflicting claims are not identical, they are not patentably distinct from each other, in part because a fusion protein of claim 15 herein is indistinguishable from a fusion protein of claim 33 herein. Thus a patent bearing claims 22 and 28-33 of the copending application permits the use of a generic intein, which may be a modified intein, in a method which otherwise includes processes, intermediate products, and thiol reagents disclosed herein and in the copending application, and patenting of the claims would constitute an unjustified or improper timewise extension of the "right to exclude" granted by a patent because it would have been obvious to one of ordinary skill in the art at the time the invention was made that the specific, *Mth* R1R1

intein, of Smith et al. is among the generic inteins of the claims 22 and 28-33 of the copending application, a species-genus relationship, and because the result of a method of claim 22 of the copending application is species of result of the method of claim 1 herein.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. §112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 2, 4, 6-12, 15-19, 21-28, 34-58 and 62 are, in part for reasons of record, rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim amendments of Paper No. 14 necessitate new grounds of rejection stated herein but this rejection is not made final because new grounds of rejection are also stated. Claims 33 and 60 cannot be included in this rejection because their subject matters cannot be determined due to the recited dependency of each claim from a sole, canceled, claim.

A. Products. While the specification discloses a fusion protein of claim 15 produced by methods of, e.g., claims 5 and 13, the specification fails to exemplify or demonstrate the preparation of either cyclic fusion proteins or modified intein products now described by claims 21-28. First, for reasons of record, the specification nowhere describes a cyclic protein according to claim 21, and provides no way to distinguish such a claimed cyclic protein from a cyclic protein not produced by a method of claim 16. In a new ground of rejection, the specification describes no "modified intein that comprises a **mutant** *Mth* R1R1 intein" having the properties claim 22 recites, i.e., a generic modified intein having within it a specific mutant intein. The specification describes no detection or isolation of mutant *Mth* R1R1 inteins; instead it describes specific, terminal, modifications of *Mth* R1R1 intein to provide the properties recited in claim 22. The specification describes no

modified intein of claim 23 because the recited modifications are not present as a result of mutations in a generic intein distinct from the modifications made in the native *Mth* R1R1 intein, and also because crucial modifications recited in the claim are not disclosed to occur in an intein but actually occur in the amino acid sequence of a flanking extein, see Figure 8 of Smith et al. Similarly, the specification describes no fusion protein of claim 24 that comprises an unmodified intein that can be cleaved to produce a previously-fused target, or partner, protein "having an N-terminal cysteine or selenocysteine"; instead, only a modified intein is described which has a recited property of, e.g., temperature induced cleavage. Likewise, the specification does not describe a "modified intein [that] comprises a mutant intein [that further] comprises a mutant *Mth* R1R1 intein" according to claims 25 and 26; instead the specification describes a modified *Mth* R1R1 intein comprising no generic, mutant, intein. Claim 27 finds no support in the disclosure of the specification because its recited modifications are not present as a result of mutations in a generic intein distinct from modifications made in the native *Mth* R1R1 intein, and also because crucial modifications recited in the claim are not disclosed to occur in an intein but actually occur in the amino acid sequence of a flanking extein, see Figure 8 of Smith et al. No plasmid of claim 28 is disclosed in the specification that comprises a nucleic acid sequence encoding a modified intein that comprises either a generic, "mutant", intein, a "mutant" *Mth* R1R1 intein" or a modified intein that possesses amino acid sequence alterations that actually occur in a flanking extein amino acid sequence, see Figure 8 of Smith et al. No product of claim 62 produced by a method of claim 58 is supported by the disclosures of the specification because the method of claim 58 is incomplete where the specification teaches that a second target protein must be cleaved from an adjacent, amino-proximal, intein that is incapable of N-terminal cleavage due to modification of its amino-terminal amino acid.

B. Methods. The specification fails to exemplify or describe the practice of methods recited by claims 1, 2, 4, 6-12, 15-19, 34-45 and 47-58 and 62. In a new rejection necessitated by amendments of Paper No. 14, claims 1, 7, 34 and 35 are rejected because the amended claim 1 recites, in the disjunctive, "fused to an *Mth* R1R1 intein or modification thereof", thus describing a method that may utilize an unmodified, native, *Mth* R1R1 intein or, alternatively, a modified *Mth* R1R1 intein. The specification, however, discloses no method utilizing an unmodified, native, *Mth* R1R1 intein and clearly teaches instead that the native *Mth* R1R1 intein must be modified in order to practice a disclosed method for ligating two target proteins, thus the alternative methods of claims 1, 7, 34 and 35 are unsupported by any disclosure in the specification. In a new ground of rejection unrelated to an amendment of Paper No. 14, claims 2, 4, 6, and 36-38 are rejected because claim 2 recites a method practiced with a first plasmid of claim 1 that must, of necessity, comprise a nucleic acid sequence already encoding either "an *Mth* R1R1 intein or modification thereof", yet "further comprises" another nucleic acid sequence that "encodes at least one first modified *Mth* R1R1 intein". Yet the specification discloses no plasmid comprising nucleic acid sequences encoding a fusion polypeptide wherein tandem or paired *Mth* R1R1 inteins are fused to a first target protein. Claim 4 is further rejected because it requires that "modified *Mth* R1R1 intein" comprise amino acid sequence modifications that do not occur in any intein amino acid sequence but must present instead in the amino acid sequence of an extein that becomes a first target protein upon cleavage of the extein-intein junction with a thiol reagent.

In a new rejection necessitated by amendments of Paper No. 14, claims 8-12 are rejected because the amended claim 8 recites, in the disjunctive, "encoding . . . at least one first intein or modification thereof", thus describing a method that may utilize an unmodified, native, intein or, alternatively, a modified intein. The specification, however,

discloses no method utilizing an unmodified, native, intein and clearly teaches instead that a native intein must be modified in order to practice a disclosed method for ligating two target proteins, thus the alternative methods of claims 8-12 are unsupported by any disclosure in the specification. Additionally, claim 8 is rejected because, in its preamble, it describes a method of making a fusion protein but the specification instead describes fusion polypeptides as the intermediate products of a disclosed method of forming a peptide bond linking separate, target, proteins. Indeed, clause (b) of claim 8 recites "ligating . . . to form a fusion protein", clearly indicating that a product is a polypeptide ligated by a disclosed method, despite the mischaracterization in the terminal recitation of "fusion protein". Claim 12 is further rejected because it requires that a "modified *Mth* R1R1 intein" comprise amino acid sequence modifications that do not occur in any intein amino acid sequence but must present instead in the amino acid sequence of an extein that becomes a first target protein upon cleavage of the extein-intein junction with a thiol reagent.

In a new rejection necessitated by amendments of Paper No. 14, claims 16-19 are rejected because the amended claims 16 and 17 both recite, in the disjunctive, "encoding . . . a first intein or modification thereof", and further recite, in the disjunctive, "encoding . . . a second intein or modification thereof", thus describing methods that may utilize unmodified, native, first and second inteins or, alternatively, first and second modified inteins. The specification, however, discloses no method utilizing unmodified, native, first and second inteins and clearly teaches instead that both first and second native inteins must be modified in order to practice a disclosed method for ligating target proteins which are the same protein, a process that produces both cyclic and polymerized products but not one or the other exclusively. The alternative methods of claims 16 and 17, which require modifying any first intein – the intein with "N-terminal cleavage activity" – by replacing a

carboxyl-terminal terminal asparagine or cysteine with alanine and require modifying any second intein – the intein with "N-terminal cleavage activity" – by replacing an amino-terminal cysteine with alanine. The alternative methods of claims 16 and 17, and those of claims 18 and 19 by virtue of dependency therefrom, are further rejected, in a new ground of rejection, because there are not separate, alternative, methods that are practiced on the basis of the disclosure. Instead, as demonstrated by the subsequent publication of Evans et al., 1999, made of record herewith, the present disclosure cannot support any method of ligation of identical target proteins that yields separate cyclic or polymerized products: to the extent that the suggested method is disclosed in the instant specification it can only result in the formation of a mixture of products and there is no disclosure herein of this outcome or of the need to separate or recover cyclic products and polymerized products from the mixture by further processes. This is clear evidence that, at the time Applicant's priority document was filed, Applicant was unable to describe a claimed method with such "relevant identifying characteristic[s]" that the public could know that the inventor possessed the invention at the time an application for patent was filed, rather than by a mere "result that one might achieve if one had made that invention". *University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Claim 19 is additionally rejected because it requires that a "modified *Mth* R1R1 intein" comprise amino acid sequence modifications that do not occur in any intein amino acid sequence but must present instead in the amino acid sequence of an extein that becomes a first target protein upon cleavage of the extein-intein junction with a thiol reagent.

In a new rejection necessitated by amendments of Paper No. 14, claims 42-57 are rejected because the amended claim 42 recites, "generating by *in vivo* synthesis, a first target protein fused to at least one first intein and a second target protein". There is no

process of "generating by *in vivo* synthesis" disclosed in the specification and there is no fusion of a "first target protein [ ] to at least one first intein and a second target protein" disclosed in the specification. Claim 58 is rejected in a new ground of rejection that is not necessitated by amendments of Paper No. 14 because the method of claim 58 is incomplete where the specification teaches that a second target protein must be cleaved from an adjacent, amino-proximal, intein that is incapable of N-terminal cleavage due to modification of its amino-terminal amino acid.

The Court of Appeals for the Federal Circuit held that a claimed invention must be described with such "relevant identifying characteristic[s]" that the public could know that the inventor possessed the invention at the time an application for patent was filed, rather than by a mere "result that one might achieve if one had made that invention". *University of California v. Eli Lilly*, 119 F.3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). The specification's treatment of the subject matter recited by the claims is considered to be entirely prospective where skilled artisans in the relevant field of molecular biology could not recognize Applicant's possession of methods utilizing unmodified first and second inteins or the possession of a cyclic product in the disclosure of the specification.

Claims 1, 2, 4, 6-12, 15-19, 21, 24, 34-41, 58 and 62 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while enabling for a method of ligating a first target protein with a second target protein or peptide comprising,

a) an initially recombinant expression in a host cell of the first target protein as a fusion polypeptide comprising the first target protein fused in frame to a carboxyl-proximal, first, modified intein, wherein the intein is modification to prevent protein splicing, and wherein a first target protein having a carboxyl-terminal proline is modified by the replacement of the proline by an alanine or a glycine,

b) isolation of the fusion polypeptide from the host cell and *in vitro* cleavage of the first target protein from the first modified intein by contact with a thiol reagent producing a C-terminal thioester on the first target protein, and,

c) contacting the first target protein with a second target protein having an amino-terminal amino acid comprising a side chain capable of a trans-esterification reaction with the carboxyl-terminal thioester of the first target protein to form a peptide bond;

as well as for fusion proteins comprising such first, modified, inteins having a modification to prevent protein splicing;

is not enabling for a method of ligating a first target protein with a second target protein or peptide comprising recombinant expression in a host cell of the first target protein as a fusion polypeptide comprising the first target protein fused in frame to a carboxyl-proximal first intein which has not been modified to prevent protein splicing. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, make and use the invention commensurate in scope with these claims.

Claims 1, 2, 4, 6-12, 15-19 and 21 contemplate methods wherein an unmodified, native, intein is employed as a first intein in a carboxyl-proximal relation to a first target protein wherein both are comprised by a recombinantly-expressed, fusion, polypeptide. The inaccurate descriptions of extein modifications as intein modifications, and the apparent descriptions of inteins within inteins, of claims 2, 4, 6, 7, 9-12, 15, 18, 19, 21, 34-39 and 62 cannot remove these dependent claims from this rejection which is based on recitations of the independent claims 1, 8, 16, 17, 24 and 58. Both the prior art of record herein, particularly the U.S. Patent No. 5,834,247 to Comb et al., and the instant specification teach that a first intein in a carboxyl-proximal relation to a first target protein must be rendered incapable of protein splicing lest the splicing reaction proceed, the intein released, and a spliced polypeptide formed, long before the initial fusion polypeptide is recovered from the host cell. In order that a first target polypeptide be available and ready for ligation in a claimed method, the requisite carboxyl-terminal thioester is provided for the first target protein *in vitro*, after the initial fusion polypeptide is isolated intact from the host cell and the first target polypeptide is cleaved from the intein by contact with a thiol reagent. There is no disclosure or teaching in the instant application that shows that an unmodified intein is available for use in a claimed method that will keep the initial fusion polypeptide intact for isolation from a host cell and the first target polypeptide ready for cleavage from the intein by contact with a thiol reagent. Each of the independent claims rejected herein embraces methods wherein the scope of the potential embodiments far exceeds the specific embodiments disclosed in the specification and the

specification and prior art do not provide guidance to allow one of skill in the art to practice the methods, as claimed, without undue experimentation.

Claims 22, 23 and 25-28 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while enabling for useful fusion polypeptides comprising modified *Mth* R1R1 inteins, modified 000 R1R1 inteins, nucleic acids encoding same, and plasmids comprising such nucleic acids, wherein the modifications comprise substitution of the amino-terminal cysteine with serine or alanine and the substitution of the carboxyl-terminal asparagine with alanine, is not enabling for useful mutant *Mth* R1R1 inteins lacking such specific amino-terminal and carboxyl terminal amino acid substitutions. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, make and use the invention commensurate in scope with these claims.

Claims 22, 23 and 25-28 contemplate products wherein a "mutant" *Mth* R1R1 intein, a nucleic acid sequence encoding such a "mutant" *Mth* R1R1 intein, a plasmid comprising such an encoding nucleic acid sequence, and a fusion protein comprising the "mutant" *Mth* R1R1 intein wherein the mutant need not be modified by either or both of substitutions of the amino-terminal cysteine with serine or alanine and the carboxyl-terminal asparagine with alanine. The inaccurate descriptions of extein modifications as intein modifications, and the apparent descriptions of inteins within inteins, of claims 23, 25, 26, and 17 cannot remove these dependent claims, or claim 28, from this rejection based on the recitation of the independent claim 22, which describes a modified intein comprising a "mutant" *Mth* R1R1 intein that is "capable of thiol-induced cleavage" but is not necessarily incapable of splicing. As explained in the preceding rejection, the disclosed method for ligation of a first target protein to a second target protein requires that an adjacent, first, intein comprised by fusion polypeptide together with its first target protein be incapable of splicing. It is well settled that 35 U.S.C. §112, first paragraph, requires that a disclosure be sufficiently enabling to allow one of skill in the art to practice the invention as claimed without undue experimentation and that unpredictability in an attempt to practice a claimed invention is a significant factor supporting a rejection under 35 U.S.C. §112, first paragraph, for non-enablement. See, *In re Wands*, 8 USPQ2d

1400, 1404 (Fed. Cir. 1988) (recognizing and applying the "*Forman*" factors). Cf., *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (citing eight factors relevant to analysis of enablement). The standard set by the CCPA, the precursor of the Court of Appeals for the Federal Circuit, is not to "make and screen" any and all possible alterations because a reasonable correlation must exist between the scope asserted in the claimed subject matter and the scope of guidance the specification provides. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 25 (CCPA 1970) (scope of enablement varies inversely with the degree of unpredictability of factors involved in physiological activity of small peptide hormone); see also, *Ex parte Maizel*, 27 USPQ2d 1662, 1665 (Bd. Pat. App. & Int. 1992) (functional equivalency of divergent gene products not supported by disclosure only of a single B-cell growth factor allele). The Federal Circuit approved the standard set by the CCPA in *Genentech, Inc. v. Novo-Nordisk A/S*, 42 USPQ2d 1001 (Fed. Cir. 1997). Where claim 28 describes a broad genus of embodiments wherein the scope greatly exceeds the few disclosed embodiments, and the specification itself does not disclose the detection or isolation of a "mutant" *Mth* R1R1 intein that is "capable of thiol-induced cleavage", there is no enablement for the claimed products.

Claims 5, 13, 29, 30 and 46 are rejected under 35 U.S.C. §112, first paragraph, because the specification is not independently enabling for the recited plasmids pMRB8A, pMRB8G1, pMRB10G, pMRB9GS, pMRB9GA, and pBRL-A. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, make and use the invention commensurate in scope with these claims.

Claims 5, 13, 29, 30 and 46 describe specific biological materials and present the issue of enablement because the specification does not disclose that the claimed biological materials are freely available to the public, either currently or upon the issuance of a patent having the claimed biological materials as subject matter. Deposits under the terms of the Budapest Treaty are, in themselves, insufficient to satisfy 37 CFR §§1.805-1.807 unless they are disclosed on the record to be freely available to the public should a U.S. patent

issue on the instant application. See, *Ex parte Hildebrand*, 15 USPQ2d 1662, 1664 (1990) (restrictions must "be irrevocably removed upon the issuance of [a] patent" since Rule 9.2 of the Budapest Treaty contains a residual requirement of secrecy). See also, MPEP §608.01(p)(C)(3). Application of 37 CFR §1.801, et seq., to any deposit, including Budapest Treaty deposits, requires that an enabling disclosure based upon such a deposit be provided by submission of a declaration or averment, either by the **assignee** or the **attorney of record** over his or her signature and registration number, that gives these two assurances:

> 1) that all restrictions on the availability to the public of the deposited material will be removed, and,
>
> 2) that the viability of the deposits will be maintained, both for the duration of the patent term or for a period of twenty years in accordance with 37 CFR §§1.805-1.807.

See, MPEP §§2405-2411.05, wherein the latter section requires an amendment to the specification that introduces specific information concerning any deposit of biological materials. Such an amendment does not constitute new matter.

The following is a quotation of the second paragraph of 35 U.S.C. §112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-4, 6-12, 14-21, 24, 25, 27, 28, 31-45 and 47-60 are rejected, many for reasons of record, under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Applicant's claim amendments of Paper No. 14 did not remedy all of the deficiencies rejected in the corresponding rejection of record stated in Paper No. 13 mailed September 19, 2002, and the claim amendments and cancellations of Paper No. 14 also raise new issues under the second paragraph of 35 U.S.C. §112. Claims 33 and 60 were orphaned by the cancellations of claims 32 and 59 in Paper No. 14 and are indefinite because they have no meaning where the claims which they had modified are non-existent. Claim 15 is also indefinite in its partial dependency from a canceled claim, claim 14.

Claims 1 and 7 are indefinite in reciting, e.g., at lines 11-12 of claim 1, "a thioester reactive N-terminal amino acid" because the specification does not define "thioester reactivity" and does not indicate that reactivity with a thioester is sufficient to result in peptide bond formation, i.e., ligation. Claims 1, 2 and 16 are independently indefinite in reciting, "having N-terminal cleavage activity", because the disclosed fusion polypeptides have no such inherent activity, by contrast with the entirely inherent and independent activity of protein splicing, and because the specification discloses that it is a class of thiol reagents which have such activity. Applicant may have intended to describe the necessary lack of C-terminal splicing capacity in a first, modified, intein. Claim 2 remains indefinite for reasons of record in its recitation, at lines 6 and 7, of "C-terminal activity", which has no meaning. However, amending the claim to insert "cleavage" would not remove a basis for rejection herein because claims 8, 16 and 17 are indefinite in their recitations of "C-terminal cleavage activity" for the same reason that claims 1, 2 and 16 are indefinite in their recitations of "having N-terminal cleavage activity": the disclosed, second target protein-comprising, fusion polypeptides do not have such independent activity, by contrast with the entirely independent activity of protein splicing, and because the specification discloses that it is the prevention of splicing which permits cleavage of a second modified intein from a second target protein. Claims 4, 6, 7, 15, 34, 35, and 36 are subject to this rejection because they depend from claims 1 and 2 but fail to clarify their intended subject matters.

Claim 6 is further indefinite because the recitation concerning the first target protein fails to limit the process of step (b) of claim 1, from which it ultimately depends, and fails to distinguish which is the "first" modified intein: the original "first", or the further-comprised "first" modified intein introduced by claim 2.

Claim 8 is independently indefinite in reciting, in its preamble, "a method for fusion", because the specification discloses that recombinant methods for fusing polypeptides - by preparing nucleic acid sequences encoding in frame fusions of coding sequences specifying heterologous polypeptides, inserting these fusion polypeptides into plasmid vectors, and then recombinantly expressing the fusion products in a host cell, are the precursors for the disclosed, and intended, see clause (b) of claim 8, methods of ligating heterologous, or identical, target proteins. While an applicant may chose describe a disclosed subject matter in alterative forms, claim 8's recitation is contrary to the disclosure. Claims 9-12, 15 and 37-39 are subject to this rejection because they depend from claim 8 but fail to clarify its intended subject matter.

Claims 16 and 17 are independently indefinite in reciting, respectively, "[a] method for cyclic fusion of a . . . protein" and "[a] method for polymerizing a plurality of . . . proteins" because the specification does not disclose that these are separate and distinct processes and, indeed, they are not. Because the specification does not disclose, or even contemplate, the separation of the different kinds of products that will result from the composite method, it is not clear that the claims to separate methods can be presented. Claims 18, 19 and 21 are subject to this rejection because they depend from claims 16 and 17 but fail to clarify their undisclosed subject matters.

Claim 21 is indefinite in reciting "by the method of any one of claim 16" because claim 16 is a singular claim, not a multiplicity of claims.

Claims 4, 12, 19, 23, 27 and 45 are indefinite because their recitations confound a terminal extein amino acid position with amino acid positions in an intein and because the recitation "Pro^{-1} – Asn^{134} to Gly-Ala mutant" confounds the relationship between the termini of the intein, where the Asn is at the intein's carboxyl terminus and the Pro is in an extein flanking the amino terminus of the intein. Use of a SEQ ID NO for the native

intein in an amended sequence disclosure may indicate a better format for expressing the intended modifications.

Claim 26 is indefinite in reciting, "said specified residue is cysteine" because neither of claims 24 or 25 from which it depends indicate that any residue is "specified".

Claim 48 is indefinite in reciting "specified N-terminal comprises a cysteine" because the term specified finds no antecedent basis In claims 42 and 43 from which it depends and because the claim concerns, if Applicant desires that it limit a preceding claim, an amino-terminal **amino acid**, e.g., "The method of claim 43, wherein the N-terminal amino acid is cysteine."

Claims 58 and 62 are indefinite in their recitations of "specified N-terminal amino acid" because the public, reading the claims, cannot know what is, and what is not, "specified".

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 703.308.0583. The examiner can normally be reached between 9:00AM-5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached at 703.308.3804. Further fax phone numbers for the organization where this application or proceeding is assigned are 703.308.4242 for regular communications and 703.308.0294 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703.308.0196.

William W. Moore
May 15, 2003